[28] As described herein, the microfluidized lysate of the present invention comprises at least one solubilized antigen of at least one *Leishmania* parasite. The microfluidized lysate preparation may further comprise a mixture of amino acids, lipids, and carbohydrates. The *Leishmania* parasite may be specifically selected for a particular reason. For example, an Old World parasite, such as *L. tropica* strain WR1063, may be used in order to detect *L. tropica* infections acquired in Southwest Asia. However, any *Leishmania* parasite, Old World or New World, may be selected by one of ordinary skill in the art, prepared, and used according to the present invention. Examples of a few *Leishmania* parasites include *L. tropica, L. mexicana, L. guyanensis, L. braziliensis, L. major, L. donovani, L. chagasi, L. amazonensis, L. peruviana, L. panamensis, L. pifanoi, L. infantum, L. aethiopica.*

[29] The *L. tropica* strain WR1063 used herein was cloned by mechanical-single cell isolation and given the designation, CL1. The microfluidized lysate preparations of the present invention were prepared under current good manufacturing practices (cGMP). Disruption of the promatigotes was accomplished by microfluidization comprising passing a parasite slurry through a chamber and disrupting the cells by the sudden release of pressure. Not only does microfluidization allow the release of membrane embedded antigens, but it provides a reproducible method for providing large volumes of preparations. Although the potency of the antigens in the microfluidized lysate preparations were not affected by prolonged storage at about 2 °C to about 8 °C, the microfluidized lysate preparations were heat-treated at about 95 °C in a water bath for about 30 minutes to deactivate the proteolytic enzymes.

[30] Specifically, a strain of vicerotropic *leishmania*, WR1063, was isolated, cloned, and then characterized as *L. tropica* by isoenzyme analysis. Other *Leishmania* parasites may be used such as *L. mexicana* as described in Example 2. A master seed lot and a production seed lot of the clone were used to initiate individual bulk production lots of promastigotes. Three bulk lots (about 1 x 10^{11} promastigotes each) were pooled in a vial and immediately placed on dry ice. The pooled promastigotes were then thawed by placing the vial in a water bath at about 56 ± 2 °C. Immediately upon thawing, the vial was placed at about 4 ± 2 °C to cool. After cooling, the contents of the vial were transferred to a pre-tared 250 ml sterile centrifuge bottle on ice using a 10 cc sterile syringe. Two 0.5 ml samples of the suspended cells were pipetted and placed in a sterile 1.5 ml Nunc cryovial (Fisher Scientific, Pittsburgh, PA) and stored at about -80 ± 10 °C.

[31] A microfluidizer, Model #M-110S was used to extract the *L. tropica* soluble proteins. The regulator was set and wet ice was put into the cooling jacket of the microfluidizer. The pump was primed by placing the inlet tubing into 500 ml of 0.001% Tween 80 and 0.9% saline and then opening the air valve. With the regulator at about 100 ± 5 psi, the inlet tubing was inserted into the promastigotes suspension and run through the microfluidizer. Because the cracking pressure fluctuates, an average reading was taken. Cracked cells were collected into the reservoir containing the uncracked cells and cracking continued for about 10 ± 1 minutes. The pre- and post-cracking temperatures were recorded. The 250 ml tube containing the lysed promastigotes was capped and stored at about 4 ± 2 °C.

[32] The cracked cells were dispensed into a sterile 250 ml centrifuge bottle and centrifuged at about 3,100 ± 200 rpm (1566 x g) in a Sorvall GSA rotor within a RC-5 Sorvall centrifuge at a time setting of about 30 ± 1 minutes and at a temperature of about 4 ± 2 °C. The bottle was removed and placed in a Class II Biohazard cabinet. The supernatant was poured of into a second sterilized 350 ml centrifuge tube and stored at about 4 ± 2 °C. The pellet was then suspended with about 20 ml of Buffer B comprising, 0.001% Tween 80 diluted with 0.9% saline, and vortexed with vortex mixer. Ice was added into the cooling jacket of the microfluidizer as needed. When the regulator was at about 100 ± 5 psi, the inlet tubing was inserted into the suspension and run through the microfluidizer in a continual fashion for about 10 minutes. The cracked cells were collected into a 250 ml centrifuge bottle containing the lysed promastigotes and placed on ice. The pre- and post-cracking temperatures were recorded. Then two 0.5 ml of the cracked cell samples were taken and stored at about -80 ± 10 °C. On ice, the cracked cells and the supernatant were combined and mixed by swirling in the container.

[33] To avoid any possibility of breakdown, the post-cracked cells were immediately centrifuged at about 12,200 ± 200 rpm (about 23,435 to about 25,062 x g) in a Sorval GSA rotor within a RC-5 Sorvall centrifuge for about 30 ± 1 minutes at about 4 ± 2 °C. Then the bottle was removed and placed in BSC, the supernatant was poured off into a sterile 250 ml graduated cylinder and the pellet in the 250 ml bottle was retained.

[34] A biosafety cabinet was sterilized as well as other items placed in the cabinet with 70% alcohol. A UV lamp was kept on in the cabinet for 15 minutes prior to use. The bulk lysate and centrifuged promastigotes were filtered. The volume of centrifuged promastigotes solution was estimated and the in process purified bulk lysate was

aseptically filtered using a 500 ml 0.22 μm Millipore filtration unit (Fisher Scientific, Pittsburgh, PA). The filtered bulk was aseptically transferred into a preweighed, sterile bottle. The bottle of the bulk was reweighed and the bulk weight was determined. Two 0.5 ml aliquots were taken aseptically and retained at about -80 ± 10 °C. One 1.0 ml sample was taken for protein concentration testing by the Bio-Rad method (Bio-Rad Laboratories, Hercules, CA) and for SDS-PAGE.

[35] The bulk clarified lysate was tightly sealed and stored at about 4 ± 2 °C until treated with heat. 25% glycerol was added to the bulk clarified lysate to give a final concentration of about 1% and then mixed by swirling. Then the total weight was determined. Then the bulk clarified lysate was incubated for about 30 ± 2 minutes using a water bath at about 93 ± 2 °C. After heat treatment, the bulk was cooled at about 4 ± 2 °C. Aseptically two 0.5 ml aliquots were taken and retained at about -80 ± 10 °C. One 1.0 ml sample was taken for protein concentration testing by the Bio-Rad method (Bio-Rad Laboratories, Hercules, CA) and for SDS-PAGE.

[36] The final clarified lysate protein concentration was aseptically adjusted to about 0.35 ± 0.05 mg/ml with about 0.4% phenol buffer. Then samples were aseptically obtained and assayed for protein content by BCA, endotoxin content LAL (gel-clot), pH, sterility, HPLC, purity by SDS-PAGE, color and appearance.

[37] An Omnispense dispensing pump (Wheaton Science Products, Millvilee, NJ) was used to dispense about 1.0 g ± 5% of the final clarified lysate into 10 ml depyrogenized, sterile, glass vials. The bulk final clarified lysate was swirled on an orbital shaker at about 60 rpm during the filling operation to ensure homogeneity. The final vials were inspected and weight checked. The vials were labeled and tested for sterility, pH, protein concentration, LAL, SDS-PAGE, HPLC and immunogenicity.

[38] The resulting microfluidized lysate preparation was used in a skin test assay for detecting whether a subject had been exposed to a *Leishmania* parasite, such as *L. tropica*. Clearly, it is well within the ability of one of ordinary skill in the art to produce microfluidized lysate preparations from other *Leishmania* parasites, such as *L. mexicana* and *L. guyanensis*, according to the method disclosed herein in order to determine whether a subject has been infected with or exposed to other *Leishmania* parasites. Additionally, one of ordinary skill in the art may prepare a microfluidized preparation from more than one *Leishmania* parasite. Thus, the present invention also provides a multivalent microfluidized lysate preparation prepared from at least two different